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Immunological signaling networks: Integrating the body's immune response^{1,2}

J. D. Lippolis³

Periparturient Diseases of Cattle Research Unit, USDA-ARS,
National Animal Disease Center, Ames, IA 50010

ABSTRACT: The role of the immune system is to protect against infection and to eliminate disease from the host. Nonimmune cells can not only act as physical barriers, but also respond to microbial stimulation to release antimicrobial molecules, whereas immune cells are primarily responsible for eliminating pathogens or cancerous cells. In addition, immune cells regulate the immune response affecting the types of cells that are activated or suppressed. The following discussion is an

overview of the immune system and its interconnection with the host. How nonimmune cells and innate and adaptive immune cells work separately and together to respond to a pathogenic challenge is discussed. In addition, how the immune system can be affected by factors such as nutrition and stress, and how the immune system can affect factors such as fertility demonstrates the integration of the immune system in processes other than elimination of pathogens.

Key words: adaptive, cytokine, immunology, innate, nutrition, Toll-like receptor

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INTRODUCTION

The immune system is a dynamic, robust, and complex system whose purpose is to rid a host organism of pathogenic organisms or cancerous cells. In addition, cells in this system form physical barriers that prevent entry of pathogens and can secrete molecules with antimicrobial actions. Together, this network of cells and molecules is in a precarious balance between action and inaction. This system is composed of numerous cells and molecules whose lethality must be potent enough to clear dangerous organisms or cancerous cells, and yet specific enough to kill without extensive collateral damage to the host. In cases when the immune system is suppressed, the host may be overcome by disease. In contrast, when the immune system is hyper-reactive, the result may be anaphylaxis or autoimmune disease, with equally lethal results. Understanding the immune system has helped in the development of therapies to boost the weakened immune system and suppress an overactive system. Research

into the immune system has yielded a constant stream of new regulatory molecules and new functions to known regulatory molecules that help control the immune system. In addition, the characterization of immune cells is continually being redefined and refined into more specific functional groups, each with a specific role to play in a response. Although the picture of the immune system is becoming more and more complicated, this research is filling important gaps in our knowledge of how the immune system functions. This knowledge has given us clues into how we may therapeutically manipulate this system.

Historically, the immune system has been categorized into 2 categories: innate and acquired immunity. Innate immunity has been defined as consisting of those functions that are nonspecific in nature and with which the host is born. Innate immunity provides the first line of defense against invading pathogens. However, some pathogens have developed the ability to escape detection or clearance by the innate immune system. Acquired or adaptive immunity is suited to the task of fighting the ever-changing pathogens and does so with a dynamic antigen pathogen recognition system. Some of the most exciting advances in immunology in the last decade have been the linking of the innate and acquired immune systems. Linkages between these 2 systems have begun to explain the initial steps in the inflammatory process, as well as the stimulation and activation of immune cells.

The complex interactions among cells that direct the immune response are not limited to immune cells. The

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³Corresponding author: john.lippolis@ars.usda.gov

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idea that immune cells are active participants in an immune response and that nonimmune cells are merely spectators is incorrect. Increasing numbers of cell types (e.g., adipose, myofibers, and epithelia) express an array of molecules that detect pathogens, express immunoregulatory cytokines, or secrete antimicrobial peptides. It may well be that all cell types can play a role in an infection. The first cells to react to an invading pathogen could be cells of the normal nonimmune tissues. The effects of nonimmune cells have been shown to be on both innate and adaptive immune cell types. For example, nonimmune cells, such as epithelial cells or adipose cells, can secrete IL-1, an activator of neutrophils (innate), or can secrete IL-15, an effector of T cells (adaptive), respectively.

INITIATION OF AN IMMUNE RESPONSE

Two thousand years ago, Celsus, a Roman physician and medical writer, described the clinical manifestations of inflammation as rubor (redness), calor (warmth), tumor (swelling), and dolor (pain). These signs indicate that the immune system is actively working to eliminate a real or perceived threat from the body. For years, researchers have worked on the problem of how the host's immune system is able to specifically detect and direct a response that will ultimately destroy a pathogen. Pioneering work, such as that of Medawar (Billingham et al., 1953), was essential to the idea of central tolerance, which is the elimination of immune cells that react toward self antigens. Thus, the concept that the immune system is able to detect and distinguish "self" from "nonself" was established. Because the adaptive immune system functions through recognition of specific antigens, central tolerance (eliminating cells that recognize self) is a concept that is limited to the adaptive immune system. If nonself is the trigger for the adaptive immune system, what is the trigger for the innate immune system? What is the trigger for inflammation? The question was answered, in part, by the discovery of cell surface receptors expressed on various cell types that recognize specific molecular patterns from pathogens.

Pattern Recognition

First described in *Drosophila* (Lemaitre et al., 1996), Toll-like receptors (**TLR**) are a family of cell surface receptors that bind to various molecules that are specific to pathogens. These receptors are some of the earliest surveillance mechanisms for the detection of infections. These receptors associate with pathogen-associated molecular patterns (**PAMP**), which are conserved motifs unique to microbes. The PAMP range from different components of bacterial cell walls, such as lipopeptides and lipopolysaccharides, to various nucleotides unique to microorganisms, such as single-stranded RNA and CpG DNA. Although the various TLR recognize a diverse list of ligands, they are germ-

line-encoded and therefore restricted in their adaptability. There are 3 categories of pathogen molecule receptors: cell surface, intracellular, and secreted (Table 1).

The TLR were the first discovered and are the most studied of all the pathogen molecule receptors. These cell surface molecules are expressed on a number of immune and nonimmune cells types. To date, 10 TLR family members have been identified in humans, with unique pathogen-associated molecular patterns (Akira, 2003). These PAMP include proteins, such as flagellin from gram-negative bacteria, which is recognized by TLR5; lipoproteins and peptidoglycan from various bacteria, which are recognized by TLR2; and various nucleotide molecules (e.g., double-stranded RNA, mRNA, single-stranded RNA, CpG DNA), which are recognized by various TLR. In addition, the specific mammalian TLR, TLR4, is partially responsible for the immune reaction initiated by lipopolysaccharide, which is a component of the outer membrane of gram-negative bacteria (Poltorak et al., 1998). These TLR can work independently or synergistically when simultaneously stimulated (Trinchieri and Sher, 2007). In addition to the TLR, there are other cell surface receptors, such as dectin-1, which bind to beta-glucan. This receptor is important for macrophage recognition and phagocytosis of cells, such as the yeast *Candida albicans* (Gantner et al., 2005). Interestingly, *C. albicans* can rapidly switch between yeast and filamentous morphologies, and only during yeast budding and separating is the beta-glucan molecule exposed to the host and recognized by dectin-1. During filamentous growth, when the host is not exposed to beta-glucan, the filamentous form of *C. albicans* plays a critical role in the pathogenesis of this microbe (Gantner et al., 2005). Thus, growth in the filamentous morphology may be an adaptation to selection pressure by recognition of pattern recognition receptors of the immune system.

In addition to cell surface receptors such as the TLR and dectin-1, similar molecules are secreted by a variety of cell types that recognize microbial molecules. One family of secreted pattern-recognition receptors is the peptidoglycan-recognition proteins (**PGRP**). In humans, 4 PGRP have been identified that not only recognize microbial components, but that also have antimicrobial activity. The PGRP have been shown to be selectively expressed and secreted by various cells and tissues, such as polymorphonuclear leukocytes (neutrophils), M cells (found in intestinal Peyer's patches), skin, eyes, sweat glands, liver, and the oral cavity (Royet and Dziarski, 2007). Bovine PGRP has been shown to kill either gram-negative or gram-positive bacteria and fungi in vitro (Tydell et al., 2002). Mice defective in one of their PGRP genes (PGLYRP-1) are more susceptible to infections of some gram-positive bacteria (Dziarski et al., 2003). In addition, neutrophils from these mice are defective in killing gram-positive bacteria (Dziarski et al., 2003). The PGRP have been shown to protect mice against an

Table 1. Pattern recognition receptor binding to various pathogen-associated molecular patterns¹

Location	Type of binding	Description
Cell surface	Toll-like receptor	A family of cell surface pattern recognition receptors that recognize various microbial products
Cell surface	Dectin-1	A C-type lectin-like receptor that binds beta-glucan.
Cell surface	CD14	Binds to lipopolysaccharide
Intracellular	Nucleotide-binding oligomerization domain-like receptor	A family of intracellular pattern-recognition receptors that bind to peptidoglycan fragments
Secreted	Peptidoglycan-recognition proteins	Secreted peptidoglycan recognition proteins whose function is both microbial recognition and as an antimicrobial effector
Secreted	Mannose-binding lectin	A C-type lectin receptor specific for the glycan region of peptidoglycan; activates complement

¹These receptors can be cell surface, intracellular, or secreted. After binding to their ligand, the pattern recognition receptors can initiate an intracellular signaling cascade that results in alterations in cytokine gene expression or can act directly as antimicrobial effectors.

experimental lung infection with *Staphylococcus aureus*. Interestingly, normal flora bacteria are resistant to the effects of PGRP (Lu et al., 2006).

An additional group of pattern recognition receptors has been described that bind to intracellular microbial molecules. These receptors, referred to as nucleotide-binding oligomerization domain (**NOD**)-like receptors, bind to various gram-positive and gram-negative bacterial molecules. In addition, the NOD-like receptor NALP3 binds uric acid, which is a molecule released from necrotic cells (Fritz et al., 2006). Therefore, the role of the pattern recognition receptors may include not only detection of microorganisms, but also detection of any injury to tissue that may result in necrotic cell death.

These pattern recognition receptors have redefined the innate immune system from a system of static barriers (e.g., skin, pH, etc.) and nondiscriminating cells that nonspecifically sample their environment to a complex system that can specifically react to unique pathogenic challenges. For example, cytokine gene expression of macrophages stimulated with ligands for TLR2 and TLR4 elicited unique responses; ligands for TLR4 stimulated more IL-1 β , interferon (**IFN**)- γ , and IL-12p40, whereas TLR2 ligands released more IL-4 and IL-5 and less tumor necrosis factor (**TNF**)- α (Akira, 2003). Interestingly, these signals in combination can act complementarily, synergistically, or antagonistically in their ability to modulate both the innate and adaptive immune systems (Trinchieri and Sher, 2007).

Intracellular Signaling

With the exception of the mammalian PGRP, which have direct antimicrobial activity, the functions of the pattern recognition receptors are to bind a specific antigenic determinate and initiate a signaling cascade that leads to an immune response. Many TLR (e.g., TLR4 and TLR2) and some of the NOD-like receptors (e.g., NOD1 and NOD2) begin intracellular signaling cascades that lead to the eventual activation of nuclear factor kappa B (**NF κ B**; Fritz et al., 2006; Parker et al.,

2007). Activation of **NF κ B** can lead to the activation of genes encoding various cytokines and chemokines that are central to an immune response. Typical to the TLR stimulation is the production of proinflammatory mediators such as **TNF**- α , IL-1 β , and IL-6. These cytokines play a role in pathogen clearance by stimulating phagocytosis and superoxide production in macrophages, stimulating differentiation and maturation of B cells and T cells, and acting as a chemoattractant and activator for cells such as neutrophils. In addition, TLR stimulation can lead to the production and release of chemokines, such as CXCL8 (IL-8) and CXCL2/3. These chemokines act by augmenting neutrophil adhesion, degranulation, and antimicrobial activity (Parker et al., 2007).

In addition to intracellular signaling through the **NF κ B** pathway, TLR can cause the activation of alternate kinases that regulate the **IRF** regulatory factor (**IRF**) family of transcription factors. Activation of the **IRF** transcription factors can lead to gene expression of various **IFN** genes (O'Neill, 2006). The molecular signaling pathways that are activated depend on which TLR is stimulated. For example, TLR2 can activate **NF κ B** and cause the gene expression of **TNF**, whereas TLR3 can activate both the **NF κ B** and the **IRF3** transcription factors, resulting in the gene expression of both **TNF** and **IFN**- β (O'Neill, 2006). As discussed above, various TLR cause the expression of different cytokines. That observation is based on which intracellular signaling pathway(s) have been activated. In addition, TLR signaling may be affected by factors such as the length of time of TLR stimulation (O'Neill, 2006).

The intracellular signaling pathways through which TLR transmit the activation signal to the transcription factors are vulnerable to interruption. Various viral proteins and glucocorticoids specifically inhibit proteins in one or more of these pathways. This raises the interesting possibility of specifically designing therapeutics for antiinflammatory treatments (O'Neill, 2006).

Intercellular Signaling

The term cytokine was originally used to distinguish a group of immunoregulatory proteins from other cel-

lular growth factors. In the general sense, the term cytokine refers to a diverse group of soluble proteins or peptides that regulate a variety of cell functions at the nanomolar concentrations. Cytokines regulate and modulate cells under both normal and pathological conditions. The term cytokine can include other immunoregulatory protein groups, such as IL and chemokines. The subgroups were given names to describe unique features of a group; however, sometimes the definitions did not hold up. For example, the term IL was originally coined to describe regulatory molecules thought to be expressed by only leukocytes and affecting only leukocytes. However, cells from adipocytes to epithelia express numerous IL, and a number of cell types, such as endothelial cells and hepatocytes, can be affected by IL.

Unlike hormones, cytokines are not made by specialized cells, but rather by a number of very diverse cell types. Likewise, there is not one specific cell type that is the sole target of most cytokines. For example, IL-1 can be produced by monocytes, macrophages, neutrophils, granulocytes, endothelial cells, fibroblasts, muscle cells, keratinocytes, osteoclasts, astrocytes, T cells, and natural killer cells. Interleukin-1 can affect B-cell proliferation and synthesis of antibodies; it promotes adhesion of neutrophils, monocytes, T cells, and B cells; it acts as a chemoattractant for leukocytes; and it stimulates the proliferation and activation of natural killer cells, fibroblasts, thymocytes, and glioblastoma cells.

Expression of cytokines is tightly regulated. In response to an infection, the expression of numerous proinflammatory cytokines is up-regulated. These proinflammatory cytokines can function as chemoattractants and induce expression of adhesion molecules, which cause responding immune cells to localize to the site of infection. In addition, cytokines can cause the functional maturation of immune cells to enable their response to or recognition of pathogens. To balance the proinflammatory cytokines, a group of antiinflammatory cytokines dampens the immune response to prevent injury to the host by its own immune system. However, at times the proinflammatory cytokines may become uncontrolled and rise to levels that are pathogenic. As Lewis Thomas stated in his book *The Lives of a Cell*, "When we sense lipopolysaccharide, we are likely to turn on every defense at our disposal; we will bomb, defoliate, blockade, seal off, and destroy all the tissues in the area. All of this seems unnecessary, panic-driven. . . . The self-disintegration of the whole animal that follows a systemic injection can be interpreted as a well-intentioned but lethal error. The mechanism is itself quite a good one, when used with precision and restraint." A properly controlled response to a pathogen will result in cytokine expression that will lead to leukocyte recruitment, antibacterial activity, and maturation of dendritic cells (DC). However, excessive expression of cytokines can lead to fever, edema, pain, tissue damage, systemic inflamma-

tory response syndrome, and possibly death (Tracey, 2007).

CELLULAR NETWORKS

As discussed above, cytokines are expressed by a variety of cell types and affect a large number of cell types. Information has been compiled regarding the cytokines that act on multiple immune cell and nonimmune cell types. The results showed that both immune and nonimmune cells are tightly linked together in a complex network of cytokine expression and response (Frankenstein et al., 2006). The cytokines expressed by nonimmune cells at the initiation of inflammation may determine the strength and type of immune response. What then is an immune cell? If we define an immune cell as a cell that can detect and respond to the presence of a pathogen, then many cell types would be included in this definition. For example, our group has shown that mammary secretory epithelia express TLR2 and TLR4 (Reinhardt and Lippolis, 2006). A reasonable hypothesis would be that TLR expressed on mammary secretory epithelial cells would be important for detecting mastitis and that their stimulation would result in cytokine secretion by these cells and subsequent recruitment of neutrophils and lymphocytes. Are mammary secretory epithelia immune cells? If we define immune cells as cells that express immunoregulatory cytokines, then adipocytes, keratinocytes, epithelia, and more could be considered immune cells. If we define an immune cell as a cell that secretes antimicrobial proteins, then keratinocytes would fit this definition (Bando et al., 2007). Regardless of the definition of an immune cell, any comprehensive study of an immune response to a pathogen will likely include a variety of immune and nonimmune cell types linked together in a complex network. In recent years, studies have begun to elucidate the interactions among various cell types.

Connections Between the Innate and Adaptive Systems

The innate immune system is a phylogenetically conserved system and is present in most multicellular organisms (Takeda et al., 2003). The classical definition of the innate immune system is an immune system built of barriers to pathogens. Protective factors, such as environment (e.g., pH, temperature, and oxygen tension), and physical barriers, such as skin and mucous membranes, are passive and therefore unable to react to pathogens in a dynamic way. Phagocytosis is also nonspecific unless the pathogen is opsonized, meaning that the acquired immune system has generated specific antibodies that coated the pathogen, thus tagging it for the phagocytic cells. The concept that the innate immune system is a nonspecific antimicrobial defense system was changed by the discovery of antigenic pattern-recognition proteins. These proteins

Table 2. Expression of Toll-like receptors (TLR) on populations of dendritic cells (DC)¹

DC type	TLR expressed	Result of TLR stimulation ²
Monocyte	TLR 1, 2, 4, 5, 6, 8	Maturation
Myeloid DC	TLR 1, 2, 3, 5, 6, 7, 8	Secrete IL-12, TNF, IL-6
Plasmacytoid DC	TLR 7, 9	Secrete type I interferons
CD8a+ DC	TLR 1, 2, 3, 6, 9	Secrete IL-12
CD11b+ DC	TLR 1, 2, 3, 5, 6, 7, 9	Secrete IL-10

¹Different types of DC express unique combinations of TLR. Additionally, the response to the same TLR stimulation can be unique depending on the type of DC. The expression of different cytokines will then affect the maturation of naïve helper T cells into one of a number of functionally unique subtypes (see Iwasaki and Medzhitov, 2004).

²TNF = tumor necrosis factor.

allow skin to be not only a physical barrier, but also an active responder when stimulated through receptor molecules such as the TLR to express cytokines. The adaptive immune system is the arm of the immune system that specifically responds to an antigen. As opposed to the innate immune system, which uses either passive barriers or receptors that recognize conserved microbial molecules, the adaptive immune system can not only specifically recognize a species of microbe, but also distinguish variants of a species. Antibodies generated by B cells recognize whole antigens, whereas the T-cell receptors recognize fragments of antigens presented by specialized molecules called major histocompatibility complex (**MHC**) class I or class II molecules. This molecule recognition mediated by either the B cell or T cell is often described as fitting like a lock and key. It has been shown that small changes in the antigen (e.g., the loss of a hydroxyl group) can result in the complete loss of recognition by the antibody or the T-cell receptor (Lippolis et al., 1995). Textbooks often describe the innate and adaptive immune systems as independent in function. However, in the last decade the interdependence of these 2 systems has been shown.

DC and T-Cell Priming

Dendritic cells are specialized antigen-presenting cells that are critical to the activation and maturation of naïve T cells. The DC initially exist in an immature form that is efficient in its ability to phagocytose but poor in its ability to present antigens to T cells. Maturation of DC causes them to express all the necessary cell surface molecules to become efficient antigen-presenting cells while the ability to phagocytose is diminished. Immature DC reside in tissues, where they phagocytose molecules in their environment, awaiting an activation signal. Upon activation, the maturing DC translocates to a regional lymph node to present antigen to naïve T cells found in the lymph node (Banchereau and Steinman, 1998). Signals received by the DC through TLR pathways play a role in processes such as migration of the DC to the regional lymph node and transformation into mature DC. Stimulation of DC with a TLR ligand induces down-regulation of the

chemokine receptor CCR6, an inflammatory chemokine, and up-regulation of CCR7, a lymphoid chemokine. This chemokine receptor expression shift alters the DC from seeking the site of inflammation to seeking lymphoid tissue, giving the DC the ability to migrate from their residence tissue to the regional lymph node (Dieu et al., 1998). In addition, TLR stimulation results in the expression of maturation markers such as CD80, CD86, and CD40. These molecules are responsible for a second signal transmitted to T cells in addition to the antigen-specific signal delivered by the MHC-peptide antigen complex, which is required for activation of the T cell. It is interesting to note that the same stimulation of 2 different subtypes of DC, the myeloid DC and the plasmacytoid DC, with the TLR7 ligand induces the cells to secrete different cytokines, IL-12 and IFN- γ , respectively (Iwasaki and Medzhitov, 2004). Thus, the subtype of DC that responds to an infection can significantly affect the type of adaptive immune response. These various DC subtypes play a role not only in the activation of T cells, but also in determining the type of T-cell response elicited. Furthermore, each subset of DC expresses a unique set of TLR (see review by Iwasaki and Medzhitov, 2004; Table 2).

Thymocytes are divided into 2 main categories, the cytotoxic T cells (**CTL**) and the helper T cells (**T_H**). Both reside in lymph nodes in a naïve state until stimulation, and both are stimulated by activated DC. The function of an activated CTL is to kill host cells infected with a pathogen, as detected by antigens expressed in association with MHC molecules on the surface of infected cells. Helper T cells have a less direct effect on the infection, but perhaps a more important role. Stimulation of mature T_H cells can cause the expression a large variety of cytokines that can direct the immune response toward a CTL-mediated, B-cell-mediated, neutrophil-mediated response, or to counter-regulate the response. When a naïve T_H cell matures, it develops into 1 of 4 types of T_H cells. Each expresses a unique group of cytokines, and each directs the immune system toward one of the above responses. The type of T_H cell is determined when the DC stimulates the naïve T_H cell by the presence or absence of specific cytokines (Figure 1). The cocktail of cytokines ex-

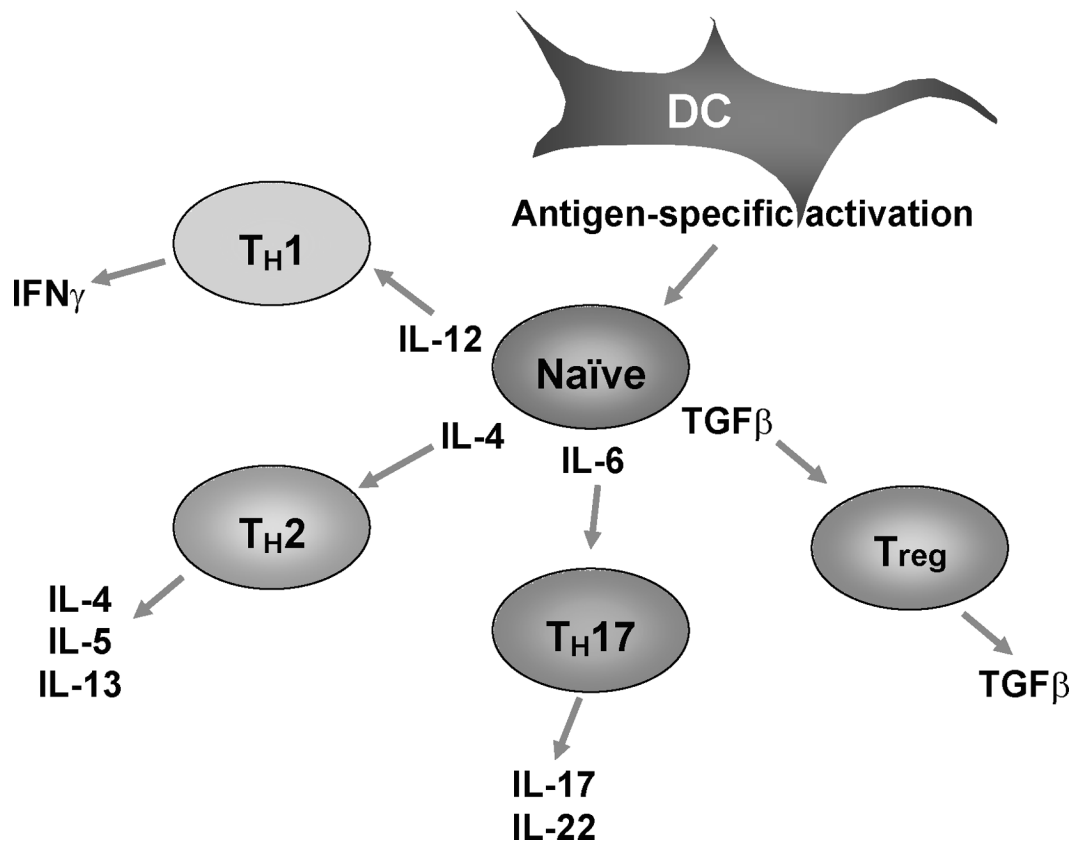


Figure 1. Maturation pathways of T helper cells (T_H). Dendritic cells (DC) from a site of infection enter the regional lymph nodes and come in contact with naïve T cells. Specific naïve helper T cells are stimulated to mature by DC that present antigen-specific epitopes. In the presence of various cytokines, the naïve helper T cells can mature into one or a variety of helper T-cell subtypes. These subtypes each have unique immunological actions, such as activation or inhibition of cytotoxic T cells, B cells, and neutrophils. IFN = interferon; TGF = transforming growth factor.

pressed by the DC differs between the various types of DC and the type of stimulation that activated the DC (Reiner, 2007). Therefore, the type of antigenic stimulation that activates the DC determines how the DC will activate the naïve T cells, and how the naïve T cells are activated determines what type of T_H cell is generated.

T_H17 and Neutrophil Recruitment

Not only does the innate immune system seem to control and direct the adaptive immune system through DC and TLR stimulation, but a new subtype of helper T cells also has been reported that stimulates the innate immune system. Recently, a subtype of T_H cells has been described that uniquely secrete IL-17, and is thus referred to as T_H17 cells (Dong, 2006). Interleukin-17 induces several innate immunity mediators, such as IL-6, IL-8, granulocyte colony stimulating factor (**G-CSF**), and PGE_2 (Bi et al., 2007). Many of these innate immunity mediators recruit neutrophils to the site of the infection. In addition, T_H17 cells secrete IL-22, which, in combination with IL-17, has been implicated in barrier function by promoting junctional integrity of the epithelia (Reiner, 2007). More-

over, the combination of IL-17 and IL-22 has been shown to synergistically induce the expression of antimicrobial peptides by keratinocytes (Liang et al., 2006). The current hypothesis is that the function of T_H17 cells is that of a mediator of the immune response to extracellular bacteria. Stimulation of T_H17 cells and their subsequent secretion of IL-17 focus the immune system toward extracellular pathogens by exerting its effect on neutrophil recruitment, epithelial barrier function, and expression of antimicrobial peptides.

Health Issues with Immunological Connections

Nutrition, stress, and reproduction are examples of generalized events or effects that can have a dramatic impact on the immune system. Previously, it was thought that the effects of these general health issues had only an ancillary affect on immune function. However, with the elucidation of more cellular and molecular immune pathways, these general health issues have started to be defined at the molecular level. Feed components, such as vitamins, directly affect gene expression in immune cells, stress causes the release of steroids that affect expression of molecules responsible to immune cell trafficking, and the reproductive sys-

tem seems to need immune cells to maintain pregnancy.

Molecular Effects on Immunity by Nutrients

The impact of nutrition on health is the subject of a significant body of research. This research has shown that nutrition can affect the ability of an animal's immune system to fight a disease. This connection between nutrition and immune function has been described at the cellular and even the molecular levels. This review is limited to the effects of 2 vitamins whose effects on the immune system have been described at the cellular and molecular levels. Vitamins are critical components in metabolic pathways. Recently, vitamins have been shown to be involved in immune functions, such as T_H -cell differentiation, lymphocyte gene expression changes, and neutrophil killing potential (Wang et al., 2005; Liu et al., 2006; Mucida et al., 2007).

Each of the T_H -cell types focuses the immune response toward a specific type of pathogenic challenge (Reiner, 2007). A recent study has shown that retinoic acid can affect which T_H -cell types are generated. In addition to responding to different types of pathogens, the various T_H -cell types are also associated with pathologies, such as autoimmune and allergy responses. For example, the T_H17 -cell type is thought to be important for the immune response to extracellular bacterial infections. However, T_H17 cells are also associated with autoimmune diseases, such as inflammatory bowel syndrome (Reiner, 2007). The bacterial flora of the gastrointestinal tract provides a unique challenge to the immune system not to react against normal gut bacteria. Inflammatory bowel disease is thought to be an immune response against the normal gut bacteria. Therefore, the question is what redirects the immune system away from a reaction against resident gut bacteria. Part of the answer to this question may be answered by the action of retinoic acid on mesenteric lymph node DC. In the presence of cytokines that drive T_H17 maturation, fewer T_H17 cells were obtained when they were stimulated by mesenteric-derived DC compared with stimulation by splenic-derived DC (Mucida et al., 2007). When retinoic acid is added, both splenic and mesenteric DC stimulation of T_H17 cells are equally inhibited. When an inhibitor of vitamin A signaling is added to both splenic and mesenteric DC, they equally stimulate a large number of T_H17 cells. Thus, vitamin A may be a critical component in the control of helper T-cell maturation in the gastrointestinal tract. Immune system dysfunction caused by a vitamin A deficiency may be explained by this mechanism (Mucida et al., 2007). Retinoic acid has also been shown to augment the inhibition of IFN- γ secretion by bovine lymphocytes caused by the addition of vitamin D (Ametaj et al., 2000). Therefore, dietary levels of vitamins A and D are important, especially because they may exacerbate immune dysfunction during the

typical immunosuppression in the dairy cow seen around the time of calving.

It has long been recognized that vitamin D deficiency causes decreased resistance to infection (Rook, 1986; Reinhardt and Hustmyer, 1987), but this action was generally thought to be secondary to the endocrine effects of vitamin D on calcium metabolism. More recently, vitamin D has been shown to have a direct autocrine effect on human immune cell functions. Thus, vitamin D affects the immune system through 2 pathways. First, the endocrine pathway affects serum calcium homeostasis. Cows generally suffer a decline in plasma 25-hydroxyvitamin D₃ [**25(OH)D₃**] around the time of calving because the calcium needs of the cow are in flux owing to the demands of milk production (Horst et al., 2005). This periparturient period has been shown to be a time of general immune suppression and leaves the animals susceptible to various diseases (Kashiwazaki et al., 1985; Oliver and Sordillo, 1988; Kehrli et al., 1989, 1990; Cai et al., 1994). Part of this immunosuppression may be due to the imbalance in calcium homeostasis during this time. Evidence has shown that more than 50% of second-lactation dairy cows are subclinically hypocalcemic (R. L. Horst, NADC, ARS, USDA, Ames, IA, personal communication). Furthermore, it has been shown that serum calcium concentrations can affect immune cell function (Kimura et al., 2006). Thus, the disruption of calcium homeostasis has a direct impact on the function of immune cells.

Through an autocrine pathway, vitamin D analogs directly affect DNA gene expression of immune cells. This is accomplished when the immune cells take up serum 25(OH)D₃ and convert it to 1,25-dihydroxyvitamin D₃ [**1,25(OH)₂D₃**], which, in combination with a nuclear transcription factor (vitamin D receptor), can bind to specific DNA sequences and affect expression of multiple genes. The autocrine pathway for immune cell regulation requires sufficient circulating 25(OH)D₃ such that activated immune cells can produce their own 1,25(OH)₂D₃ in their local environment at cell concentrations that activate key pathways that would not be activated by circulating endocrine-produced 1,25(OH)₂D₃. Screening of human and mouse genomes revealed more than 3,000 genes with a vitamin D response element to which 1,25(OH)₂D₃, in combination with the vitamin D-binding protein, affects gene expression (Wang et al., 2005), some of which are involved in immune cell regulation. Additionally, stimulation of the TLR was shown to induce the 1 α -hydroxylase enzyme that catalyzes the conversion of 25(OH)D₃ to the active 1,25(OH)₂D₃. The production of 1,25(OH)₂D₃ was, in turn, necessary for the induction of antibacterial genes, such as cathelicidin (Liu et al., 2006). It was further demonstrated that lower serum concentrations of the precursor 25(OH)D₃ were correlated with a decreased ability of monocytes to kill bacteria (Liu et al., 2006). Thus, stimulation of immune cells with a TLR ligand in the presence of

25(OH)D₃ resulted in the gene expression of additional products important for the antimicrobial response, and the lack of a sufficient level of 25(OH)D₃ had a negative impact on the immune response. Use of 1,25(OH)₂D₃ as an adjuvant has also been reported, and treatment of cows with 1,25(OH)₂D₃ along with the *E. coli* J5 vaccine was shown to result in greater levels of antibodies against *E. coli* J5 in milk and serum compared with the *E. coli* J5 vaccine alone (Reinhardt et al., 1999).

Stress

The causes of stress in animals are as varied as its manifestation. Types of stress include heat, negative energy balance, transportation, pregnancy, and the mixing of unfamiliar animals. Some ways that an animal will manifest stress are in the forms of sickness and failure to thrive. Recently, these very general manifestations have begun to be defined on a cellular and molecular level. Various immune cells, such as neutrophils, T cells, and DC, are affected when an animal is stressed, and expression of specific molecules, such as CD62L (L-selectin), is affected during stress (Burton and Kehrli, 1995; Burton et al., 1995, 2005).

The initiation of a stress response involves activation of the hypothalamus, pituitary gland, and adrenal gland to release hormones such as cortisol, epinephrine, and norepinephrine. This response is known to have a dramatic effect on the immune system. For example, chronic stress in pigs caused by mixing unfamiliar animals resulted in subordinate pigs having significantly fewer white blood cells compared with the dominant animals (Sutherland et al., 2006). Furthermore, it has been established that animals subjected to restraint stress fail to mount a normal immune response that can result in failure to mount a protective immune response subsequent to pathogen challenge (Anglen et al., 2003).

The molecular mechanisms that explain the effects of stress are a subject of current research. Several groups have used gene expression microarray analysis to determine the genes affected by stresses, such as thermal stress (Collier et al., 2006), food deprivation (Ollier et al., 2007), and treatment with stress hormone, such as cortisol (Burton and Kehrli, 1995; Weber et al., 2001; Burton et al., 2005). One of the most well-studied molecular effects of stress on the immune system is the effect of cortisol on the expression of the protein CD62L, which is expressed on the surface of immune cells, such as neutrophils, and is necessary for the transmigration of the cell from the vasculature into the tissue at the site of an infection. Cortisol causes the loss of CD62L expression on neutrophils, and thus the loss of the ability to migrate through the vascular endothelium. This loss of neutrophil response is correlative with increased susceptibility of the animal to mastitis (Burton et al., 1995).

Reproduction

The immune system is significantly affected during pregnancy. There are significant interactions between the immune system and cells and tissues of the reproductive system, which are critical for the maintenance of pregnancy but are responsible for the immune suppression that is associated with increased risk of disease.

One example of the importance of the immune system to reproduction is illustrated by the interaction between leukocytes and the corpus luteum (Pate and Landis Keyes, 2001). The corpus luteum is the remnant of the ovulatory follicle. Its function is to produce progesterone, which is essential for the maintenance of pregnancy. In the absence of an embryo, the corpus luteum regresses, and this regression is initiated by uterine release of PGF_{2α}. Regression of the corpus luteum will allow a new follicle to ovulate. Interestingly, both macrophages and T cells are found in the corpus luteum. During luteal regression, the number of lymphocytes and macrophages in the tissue increases by both recruitment of cells and proliferation of resident cells (Bauer et al., 2001). Cytokines thought to be expressed by these luteal immune cells have the ability to inhibit progesterone synthesis by the bovine luteal cells and cause apoptosis of these cells, and thus regression of the corpus luteum (Pate and Landis Keyes, 2001). The exact mechanism by which the immune cells are signaled to work actively toward regression of the corpus luteum is the subject of much research. Understanding this mechanism may help in the generation of new methods to increase fertility in domestic animals.

During pregnancy, cells of the immune system undergo significant alterations that have yet to be thoroughly investigated. For example, stimulated neutrophils from pregnant women showed significantly less respiratory burst activity compared with those from a control group (Crouch et al., 1995). Similarly, 2 enzymes in the hexose monophosphate shunt, which is part of the pathway that produces the reduced nicotinamide adenine dinucleotide phosphate required for respiratory burst activity, are localized to different subcellular areas in neutrophils from pregnant vs. nonpregnant women (Kindzelskii et al., 2004). Finally, subcellular location of myeloperoxidase, an enzyme critical to oxidative burst, is altered in nonpregnant women (cytosol) compared with pregnant women (external to the cell and associated with the cell membrane; Kindzelskii et al., 2006). These alterations in neutrophil functions associated with antimicrobial activities indicate significant perturbation of the neutrophil cellular functions as a result of pregnancy. These observations support the long-held idea that immune suppression is an important mechanism in the maintenance of pregnancy and that a breakdown of the suppression is a factor in spontaneous abortions (Vince et al., 2001).

The periparturient period is a nexus of physiological events that combine to have a profound effect on the immune system. Periparturient immunosuppression is manifest in a wide range of immunological dysfunctions, including impaired neutrophil and lymphocyte functions (Kehrli et al., 1989; Shuster et al., 1996; Mehrzad et al., 2001). As part of the innate immune system, the neutrophil is an essential first responder to infection and is considered vital to effective clearance of bacteria from the mammary gland of the dairy cow (Mollinedo et al., 1999; Smith, 2000; Paape et al., 2003; Zychlinsky et al., 2003). Neutrophils have various killing mechanisms to destroy pathogens (Smith, 2000; Segal, 2005). Upon encountering invading bacteria, neutrophils will ingest the bacteria into phagosomes that are fused with lysosomes. This process stimulates neutrophils to produce large amounts of oxidizing agents in a process referred to as the respiratory burst, in which oxygen radicals are generated that serve as precursors to various antimicrobial oxidants. In addition to oxidizing agents, neutrophils contain numerous antimicrobial proteins, such as cathelicidins, hydrolases, proteases, lactoferrin, and lysozyme within granules. These proteins are either released into phagosomes to destroy ingested pathogens, or the granule contents are released out of the cell. These neutrophil functions are suppressed at and around the time of parturition (Kehrli et al., 1989; Shuster et al., 1996; Mehrzad et al., 2001). The molecular causes of periparturient neutrophil functional suppression are an area of intense research by this and other research groups.

In summary, the immune system is a complex system that enables the body of the host to be protected against or to eliminate pathogens. This system is made up of numerous cell types whose functions are still matters of investigation. This system relies not only on cells defined as "immune cells," but also on nonimmune cells to detect and respond to various infectious agents. In fact, the initial signal that begins an immune response is likely a nonimmune cell that detects pathogens through its pattern recognition receptors, such as the TLR. Stimulated nonimmune cells of various types are known to be able to secrete cytokines that can initiate an immune response.

Understanding of an immune response must not only take into account the functions of the immune cells, but also the effects that various pathogen-stimulated nonimmune cells have on the immune response. Conversely, an immune response can have important effects on the cells, tissues, and the whole host. The immune response can have negative impacts, such as those that are normally associated with uncontrolled inflammation (e.g., fever, edema, pain, tissue damage, and potentially death). In addition, constant immune stimulation will lead to suppressed growth of an animal because energy and nutrients go preferentially to immune and homeostatic pathways (Spurlock, 1997). This illustrates the important connection between the

general health and growth of an animal and the immune system. This connection has been shown not only at the whole-animal level (e.g., growth), but also at the molecular level. Dietary components, such as vitamins, have been shown to affect gene expression of a number of immune cells. Thus, the molecular pathways that tie growth, nutrition, and immune responses together are being elucidated.

The immune system is affected by various nonpathogenic stimuli and has an important role in processes other than disease control. For example, the immune system plays an important role in the maintenance of the corpus luteum. Therefore, the immune system plays an important role in reproduction. In addition, nonpathogenic stimuli such as stress can, after prolonged exposure, have a suppressive effect on the immune system and make the animal susceptible to infection.

To achieve the goal of generating therapeutics that prevent or cure diseases, we must have a better understanding not only of the mechanisms and functions of the immune system, but also of how that system is integrated into the whole host. For a vaccine to have the greatest potential for success, the animal's immune system must be working at optimal levels. Therefore, optimal diets must be given to ensure proper immune function, and stresses must be reduced to eliminate suppression of the immune response. There is likely no single treatment that will make animals disease free, but a comprehensive plan to address the various aspects of the overall health of an animal will optimize the immune system and increase the likelihood of a successful immune response.

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